VAN CLEAVE

Structure and relationship of an echinorhynchus from the gizzard-shad (Dorosoma cepedianum)

Zoology

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1910

STRUCTURE AND RELATIONSHIP OF AN ECHINORHYNCHUS FROM THE GIZZARD-SHAD (Dorosoma cepedianum)

BY

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

Harley Jones VanCleave

ENTITLED The Structure and Relationship of an Echinorhynchus from the Gizzard-Shad (Dorosoma cepedianum).

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE

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In Charge of Major Work

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Recommendation concurred in:

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Acknowledgements.

The ectoparasitic worms of the order Acanthocephala, though having received considerable attention among European investigators for more than a century, culminating in the monumental works of Hamann (1890), and Kaiser (1893), have been almost entirely neglected in this country. A few scattered references are all that we have to indicate the work that has been done on these forms in America. Though occurring most frequently in the digestive tract of aquatic vertebrates they are by no means limited to these hosts but occur in various other vertebrates, including man. In size they range from very minute animals to a length of six hundred and fifty millimeters (Giganthorhynchus gigas). These extreme variations in size are paralleled by as great variations in the shape of the body and character of the proboscis. Yet there is enough of a general likeness in the different species that we do not hesitate to include them all in the same order.

Although until recently the Acanthocephala have been quite generally accepted as belonging to the Nemathelminths, as a matter of fact they have very little in common with that group. The body musculature, especially the circular muscles, present a condition much more closely approaching that found in the Cestodes than the muscular system typical of the Nematodes. The absence of any specialized digestive tract also makes the possibility of their relationship with this group dubious. Cholodkovsky has recently summarized the points of difference which lead him to consider this order more closely related to the Plathelminths than to the

Nemathelminths. He expresses his belief that the Acanthocephala have arisen fromthe same ancestral form as have the Cestodes.

The member of this group upon which the present work has been carried on is a small Echinorhynchus averaging less than 2.5 m.m. in length. The host is the Gizzard-shad (Dorosoma cepedianum)- a fish quite common in the Illinois river near Havana, Illinois where my material was taken during the months of November and December. The small intestine, and more especially the numerous intestinal caeca, are the regions in which the infection occurs. The extreme mortality and softness of these fish make it impossible to keep them alive any length of time after capture. For this reason the freshly caught fish were packed in ice and shipped directly to Urbana where the examination for parasites was made immediately upon their arrival.

In the forty hosts examined no parasites other than Echinorhynchus were found in any case. Two representatives of this genus appeared,-one in quite large numbers, the other only in rare instances, with a total of four specimens. The study of the former species constitutes the basis of the present paper. The range of the infection varied all the way from a few individuals in most instances to as high as seventy-six in one fish. The six animals in which no parasites were found were all very small,immature, specimens.

The Looss method of fatigueing the parasites by shaking the slit open intestine in normal saline proved very satisfactory with this form,giving perfectly relaxed worms. After thorough shaking a few minutes, the killing fluid was added. For this purpose corrosive acetic solution is very valuable,since it precipitates the mucus with which the parasites are surrounded, leaving the

parasites free when the precipitate is decanted off.

In using fluids where it became impracticable to fix the entire intestine,as is necessary in the foregoing method, gentle pressure on the mass of caeca served to free many of the worms which could then be transferred to the killing fluid.

For general anatomical features glycerine jelly mounts, both stained and unstained, were in many respects superior to mounts inbalsam, for in the latter instance there is a marked tendency for the clearing to proceed too far and leave the material too transparent. However,stained balsam mounts proved of great value in the study of the nuclei and for the general location of many of the finer structures.

For detailed study of thin sections Iron-haematoxylin proved itself as indispensible here as in other lines of cytological work. Acid-fuchsin countered with Lyon's- blue to which a little picric acid is added gives an extremely good red nuclear differentiation with a light gree n cytoplasmic stain.

External Features.

The two sexes differ but little in external characters-size and shape of the posterior end of the body being the most evident points of difference. The female may slightly exceed 3.25 m.m. in length, with an average length of 2.5 m.m.,while the males average about 2.22m.m. and only in rare instances reach 3.m.m. The posterior end of the body of the female is blunt and roundded (0.147 m.m. across). In the same region of the male we may find either of two conditions present. In case the copulatory apparatus is drawn up within the body cavity the end of the body presents an invaginated appearance (Fig.2) with an average breadth of0.087 m.m. On the other hand,if the copulatory apparatus is protruded

we find a wide mouthed funnel-shaped termination, the bursa, which surrounds the cirrus.

In general form the body is slightly bowed or crescentic, the animal being concave on the ventral side. With preserved material the body is typically cylindrical and smooth except in some cases, especially in the anterior part of the more or less contracted individuals, where the appearance of segmentation previously referred to, is displayed.

Anteriorly the body ends in a bulbous proboscis (Pr.Fig. 1.), an organ found in all members of this order, but differing extremely in shape and size in the different species. In this form the proboscis is short and stout, armed with three circular rows of hooks, of which each row contains twelve. The hooks of the terminal and basal rows are opposite, while those of the median row alternate in position with these.

The hooks in this species are rather small. The length varies considerably in the different rows on the same individual. However, the difference in the length of corresponding hooks on different individuals is very slight. Spines of the greatest length (0.021 m.m.) occur in the basal row. These are also much straighter than are the hooks in the remaining rows. Next in length (0.017 m.m.) come those of the terminal row. We find in this row the most strongly recurved thorns. Shorter than the hooks of either of the foregoing groups are those found in the middle row where the length is about 0.015 m.m.

Turning now to the structure of the body wall of this organism we see that it is composed of four chief layers. Externally it is limited by a very thin translucent, slightly staining cuticula, ranging from about 0.002 to 0.005 m.m. in thickness.

Beneath this lies the subcuticula which composes the greater mass of the body wall. The structure of both of these tissues has been worked out in great detail by various authors. A dorsal and a ventral canal traverse the subcuticula longitudinally. Numerous smaller circular canals communic-ate with these main trunks at frequent intervals.

Next inside the subcuticula lies the circular muscle tissue, while inside of this come the longitudinal muscle fibres. The structure of these muscles is very peculiar. A group of several fibres is supplied with a single nucleus which does not appear in the fibres themselves, but stands out more or less distant from them. Another peculiarity of these muscle nuclei is their arrange- ment. They are arranged in two longitudinal parallel rows, one row appearing on each side of the dorsal region of the body, a little lateral to the dorsal canal.

The structure of the proboscis differs considerably from that of the body proper. The muscular system in particular is completely modified. We find no fibres in the wall as we have just noticed in the wall of the body proper.Extending backward from the tip of the proboscis is a large muscle occupying a central position in that organ. This muscle serves to draw in the end of the pro- boscis, bringing the spines all in a position with the points pro- jecting anteriorly so as not to interfere with the retraction of the proboscis within the body.

The proboscis-sheath (Pr.sh. Fig.1.) is a special adapt- ation for receiving the proboscis when thus retracted. At the base of the proboscis-sheath is located the brain (Br.). In this species the brain is a conical mass of cells from 0.08 to 0.1 m.m. long with the base attached to the posterior ventral wall of the sheath.

Backward along the side of the proboscis-sheath extend two slender cylindrical pouches, the lemnisci (l. Fig.1.) . These take their origin at the point of insertion of the proboscis with the body, and extend backward from one fifth to one fourth the length of the body cavity. Cross sections show these organs to be hung in a muscular sheath which extends around the dorsal side of the proboscis-sheath giving the lemnisci lateral positions in the body.

Two ligaments (S.l. Fig.1.) taking their origin at the base of the sheath run backward through the body cavity forming sort of a suspensor in which the genital organs are supported. In the mature female this tends to hold the eggs and egg masses together in the center of the body as well as forming a support for the genital tract. In the male these ligaments pass backward along the sides of the testes to the posterior end of the body where they become attached to the body wall.

With a few exceptions the gential organs of both sexes are very similar to those of other species of Echinorhynchus. Two testes (T-T' Fig.1.) in the male occupy the anterior region of the genital tract. These are arranged one behind the other. They are seldom of the same size- the anterior one being usually the longer and narrower of the two. The testes do not present the appearance of solid compact masses. Each one is composed of numerous small groups of cells in various stages of maturation. These small masses (0.02 or 0.03 m.m. in diameter) are separated from one another by irregular clear lines and areas.

A vas deferens leads from the anterior end of each testes passes along it s dorsal side and empties into a wedge shaped vesicle at the posterio-dorsal edge of the hind testis. From here

a duct leads to the ventral side of the body and proceeds backward along the ventral edge of the so called cement gland. About the middle of this gland occurs another dilation of the duct. From this point the duct passes backward into the seminal vesicle (S.v. Fig.2.) which communicates through the vas deferens (V.ef. Fig.2.) with the small conical cirrus. The complete copulatory apparatus of the male consists of the protrusible bellshaped bursa, (B. Fig.2.) previously mentioned, in the centre of which the cirrus (C.) is located. The function ascribed to this bursa is that of a clasping organ used during copulation.

The socalled cement-gland (Cg. Fig.2.) which has already been located as posterior to the testes, presents a radically different aspect from the homologous organs in most other species of Echinorhynchus as described and figured by various authors. The first and most obvious difference is that instead of six or more small independent glands, each with its own duct, we have in this species one large gland containing eight large nuclei. Hamann has found in the larval condition of E. polymorphus six large nuclei, each surrounded by a transparent membrane, occupy the position later taken by the cement glands. In regard to the subsequent development of these cells we quote- "Jede der groszen Zellen teilt sich in eine weitere Anzahl und die Drüse besteht in kurzen aus einer Anzahl von Zellen, die untereinander wohl abgegrenzt sind." It might thus appear that in this case, where we have a single large gland with a small number of large nuclei, that we may have a larval condition retained in the fully developeed form.

A spherical gland (G ?. Fig.2.) located just posterior to the cement gland, I have so far been unable to homologize with

any structure in the other species that have been worked upon. In stained sections this gland gives an entirely different appearance from any other structure associated with the genital system. It appears as a sac containing a mass of deeply staining granular material. The wall of the sac is composed of two distinct tissues, an outer deeply staining coat surrounding a thicker, in most cases non-staining layer. The mass within the gland takes an extremely intensive eosin stain. Acid fuchsin also stains it very deeply. The duct leading from this gland to the vas eferens (V.ef. Fig.2) is filled with a substance which takes the stain in the same degree.

The genital ganglion (G.g. Fig.2.) lies just at the anterior edge of the dorsal shield of the bursa. It is quite irregular in shape and is composed of quite a number of small cells massed together.

The mature female genital organs (Fig.3.) display very little save a complicated tract along which the ova pass in their exit from the body. Three divisions in this tract have been recognized by Saefftigen - the uterus (ut.), the oviduct (H.), and the bell or Glocke (Gl.). The uterus extends from the genital opening to the convoluted portion of the oviduct. Anterior to the latter region is a broad mouthed bell-shaped portion with two lateral horns continuing forward in the suspensor ligaments.

About midway between the two ends of the uterus is located a well developed sphincter (B.).

A long strip of muscle (M.s.) runs from the oviduct backward to the extremity of the body.

Two fan shaped sheets of tissue (S.s.) with their broad edges attached to the body wall at the sides of the mouth of the

uterus, extend backward to the dorsal wall of the body cavity.

The ova in a mature female are usually massed together in spherical or elongated bodies. There is no constancy in the size of these masses. In a single individual they range in size around an average of about 0.06m.m. by 0.05m.m. In later stages of development these masses break up and the embryos appear as spindle shaped bodies of fairly constant size (0.036 by 0.01 m.m.)

The problem of the constancy of location and numbers of nuclei is one upon which very little has been done. Because of the smallness of the entire organism and small number of nuclei present ,this species of Echinorhynchus affords quite favorable material for such a line of investigation. This phase of the work was first suggested by the remarkable regularity of position and number of nuclei found in the subcuticula of this animal. There are but six nuclei in this entire tissue. Five of these (Ec.N.1-5, Fig.1.) occur in the dorsal canal- two near the anterior end and the remaining three near the posterior end. The sixth (Ec.N.6) - the largest of the series- lies in the ventral canal in the anterior portion of the body. It is interesting to note that all six of these are found in the median sagittal plane of the body.

Of the ninty specimens examined the foregoing statement as to the number and arrangement held true in every instance save one. A small female nearly 0.2 m.m. shorter than any other individual of these examined has one of the anterior dorsal nuclei wanting; but an additional nucleus is found on the ventral surface of the body. Since Hamann and various other authors claim for the early stages of these nuclei the power of amoeboid motion,

it is not difficult to see at least how it is possible that since the two canals are connected by cross canals, a migration from one side of the body to the other is perfectly possible. It may be that some such a condition of wandering is more or less prevalent during a certain period of the development before the regular positions are taken up by the nuclei. If the nuclei are amoeboid in the early stages the wonder is that we do not find such misplacements more frequent in the mature forms.

The chromatin of these nuclei is gathered together in an alveolar mass, surrounded by a clear, non-staining cytoplasm. I have as yet been unable to find any trace of cell walls in the subcuticula. The nuclei are bounded by quite definite nuclear membranes. Stain is not necessary to bring out these nuclei for they are easily visible in cleared specimens. But in order to leave less room for doubt as to the exact number and position most of the material used for the study of this tissue consisted of stained toto-mounts and sections.

As early a worker as Dujardin reports a definite number and arrangement of these bodies in the subcuticula, even though he does err in interpreting them as "pores" or "orbicular discs". The species he describes (E. tuberosus) had five or six of the "pores" on the convex side and one on the concave side.

Both Hamann and Kaiser in tracing the development of the subcuticular tissue have observed in the early larval forms that quite a number of small nuclei are scattered throughout the tissue. Later by a process of fusion these nuclei unite to form several "giant nuclei" typical of only the larval stage. Still further development consists in the splitting up of these "giant nuclei", leaving numerous small nuclei scattered irregularly

E. clavadceps, a form in which the large nuclei resulting from the syncitium and fusion of the nuclei are retained in the sexually mature individuals, Hamann cites as a case of phylo-paedogenesis. The question arises as to whether it is better to consider it thus or as more probably the retention of certain larval characteristics in the adult. Evidence in the species upon which the present work has been carried on seems to indicate the validity of the latter position rather than present any evidence of the development of mature sexual organs in the larval stage.

Hamann records a single large nucleus in each lemniscus of E. clavula, as does Graybill for E. thecatus. The same condition holds equally true for this species.

In the proboscis there is an interesting arrangement of the nuclei. The muscle extending backward from the tip of the proboscis contains two large oval nuclei arranged one behind the other. They differ slightly in size, the hind one being the larger. At the base of the terminal row of hooks are located the remaining nuclei of this organ. A ring of twelve small, round nuclei occupy positions one opposite the base of each of the hooks. The entire nucleus does not exceed 0.007 m.m. in diameter while the chromatin mass averages about 0.005m.m. Up to the present time it has been impossible to trace any direct connection between these nuclei and the hooks opposite them. The origin of hooks, spines and similar structures in many animals from definite basal formative cells might lead us to raise the question, "What is the possibility of some such relationship in this instance as well?" Evidence to sustain either one side or the other of this question is left for the future to bring forth.

The small size and great number of the cells of which the brain is composed make that an extremely difficult region in which to determine the number of cells. At the present time this organ has not been worked over thoroughly enough to give any definite results.

Certain regions in the male display a definite, constant cell content with great clearness. The so called cement gland (C.g. Fig.2.) is a good illustration of this condition. As has been mentioned previously, this organ contains eight large nuclei, each consisting of a central chromatin mass surrounded by a halo of non-staining cytoplasm. These nuclei are embedded in a mass of loose tissue containing numerous open spaces and small ducts throughout. I find Graybill in his description of E. thecatus (Linton) records for this form a similar condition of the cement gland, - "Cement glands in compact mass, eight in number."

There is a strip of muscle (M. Fig.2.) running from the dorsal shield of the bursa to the spherical gland located between the cement gland and the seminal vesicle. On the dorsal side of this , a short distance from its attachment to the bursa we find two small nuclei (M.n.2.), taking lateral positions. Just ventral to the anterior insertion of the same strip of tissue is another pair of small nuclei (M.n.1.).

Anterior to the seminal vesicle, along side the vas deferens lie two rounded nuclei (V.def.nu.), which appear to be very closely associated with the vas deferens.

Several other regions of the male in a few instances have given evidence of constancy of cell elements, yet the evide-ence in the material at hand has not been sufficient to warrant the formulation of any general rules of occurrence.

Each of the triangular sheets of tissue extending from the mouth of the uterus in the female is supplied with a single round nucleus (A. Fig.3.) located near its ventral edge.

Both anterior and posterior to the sphincter of the uterus are located a pair of nuclei which are lateral in position (F.-G. Fig.3.)

Dorsal to the sphincter a single considerably elongated spindle shaped nucleus is to be found. (E. Fig.3.)

A single unpaired oval nucleus is embedded in the muscle strip leading from the oviduct to the posterior end of the body (D.).

The anterior prolongations of the bell have two large nuclei (C.C´) located each in the centre of a clear non-staining mass of tissue.

The coiled portion of the oviduct is supplied with several nuclei, but the complications of the coils make their exact location and relative position very difficult to determine.

While several problems regarding the anatomy of this form have not been touched upon, they are for the most part of such a nature as to require a knowledge of the early stages of development which for this species have never been worked out.

At the beginning of this paper attention was called to the position the order Acanthocephala, with its largest genus Echinorhynchus, occupies in its relations to the other parasitic worms. The accompanying table compiled chiefly from the descriptions of species in Kaiser's monograph, while in nowise intended to establish the form under discussion as a new species, is introduced here to indicate the nature of the specific characters

found in this genus. The catalog of forms given by Diesing in his Systema Helminthum shows that this species is not so unique in size as the c omparison with the species described by Kaiser seems to indicate

	Length in mm		Width in mm		Hooks		
	♀	♂	♀	♂	circular rows	longitudinal rows	length in μ.
E. moniliformis (Bremser).	60-80	40-50	1.5 - 2	1.5 - 2	10 - 15	14	13 - 17
E. angustatus. (Rudolphi)	8-17	4.5- 8	1-1.4	1-1.4	16 - 18	14	100 - 110
E haeruca (Rudolphi)	40-60	7±	2-2.2	1- 1.2	8 - 12	20	48-62.
E. trichocephalus (R Leuckart).	50-80	50-80	0.5-0.8	0.5-0.8	20	19-20	50 - 112
E. uncinatus (Kaiser).	40-60	40-60	1-1.2	1.-1.2	16- 18	18	65- 98
E. porrigens (Rudolphi).	18-28	12-22			12- 14	20	78-98
E. strumosus (Rudolphi).	3.5-4.6	3.5-4.6	0.85-0.98	0.85-0.98	10 - 14 10 ± 11	18	48 ± 84 14 - 17
E. spinosus (Kaiser).	40-60	30-45	1.5	0.9-1.1	18 - 24	30	14- 15 48-73
E. thecatus (Linton)	11- 26	7- 12	0.8-1.4	0.6-0.95	24- 31	12	48-84
E. (sp)	1.6-3.3	1.7-3.	0.27-0.5	0.17-0.43	3	12 in a row.	12-22.

Reference has already been made to the departure from the typical condition in the structure of the cement gland. However this condition is not confined to this one species. In speaking of the cement gland in E. haeruca Hamann states, "Die einzelnen Zellen sind mit einander verschmolzen und die Kerne liegen regellos zerstreut". I have also noticed the same condition in an Echinorhynchus (probably E. thec atus) taken from the intestine of the black bass.

Since the very early workers on Echinorhynchus much emphasis has been placed upon the differences in number and arrangement of the hooks on the proboscis as constituting characters of

true specific value. The quincuncial arrangement of the hooks is very clearly displayed in this species. The shape of the proboscis itself differs considerably from the form commonly met with in this genus. This short, thick, bulbous organ does not possess the numerous rows of hooks characteristic of so many species.

The work upon this problem has been carried on under the direction of Dr.H.B.Ward, to whom the author is greatly indebted for many suggestions and assistance in various other ways, including access to his excellent private library. The kind assistance of Mr.George R.LaRue,especially in matters of technique, has also been very greatly appreciated.

Figure 1.

Drawing of an entire male, magnified 531 diameters.

D. Dorsal side

V. Ventral side.

A. Anterior end.

P. Posterior end.

Pr. Proboscis.

Pr.sh. Proboscis sheath.

Br. Brain.

Ec.N.1-6. Subcuticular nuclei.

L. Lemnisci.

T.-T. Testes.

S.L. Suspensor ligament.

C.G. Cement gland.

G ?. Gland of undetermined function.

B. Bursa.

C. Cirrus.

S.v/ Seminal vesicle.

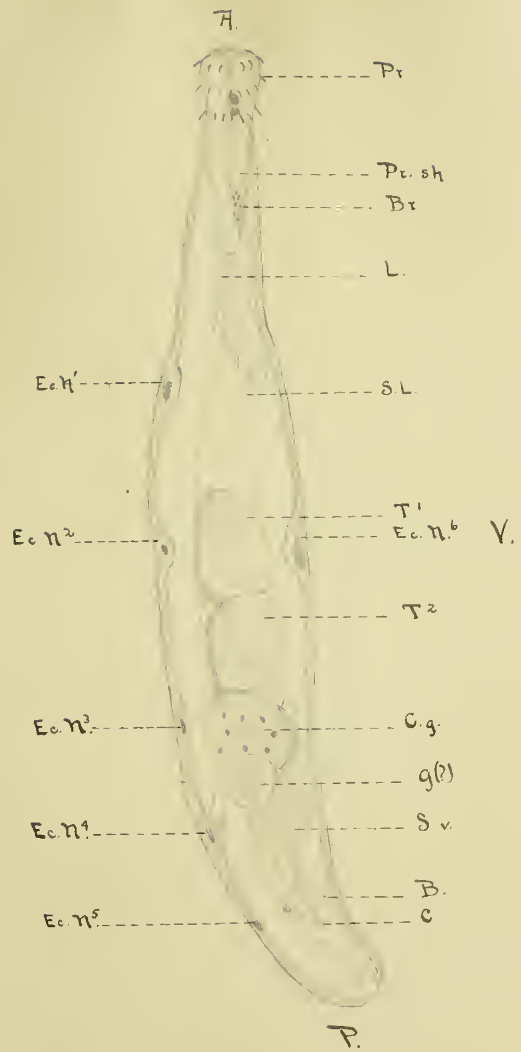

A.
Pr
Pr. sh
Br
L.
Ec. N'
S.L.
T'
Ec n 2
Ec. n. 6
V.
T 2
Ec. n 3.
C. g.
g(?)
Ec. n 4.
S v.
B.
Ec. n 5
C
P.

Figure 2.

Posterior end of male (reconstruction from sections) magnified 333 diameters.

B. Bursa.

C. Cirrus.

C.c. Circular canals in cross section.

C.g. Cement gland.

G.?. Gland of undetermined function.

G.g. Genital ganglion.

G.d. Duct from G.?.

M. Retractor of bursa.

M.n.1. Nucleus at anterior end of M.

M.n.2. Nucleus at posterior end of M.

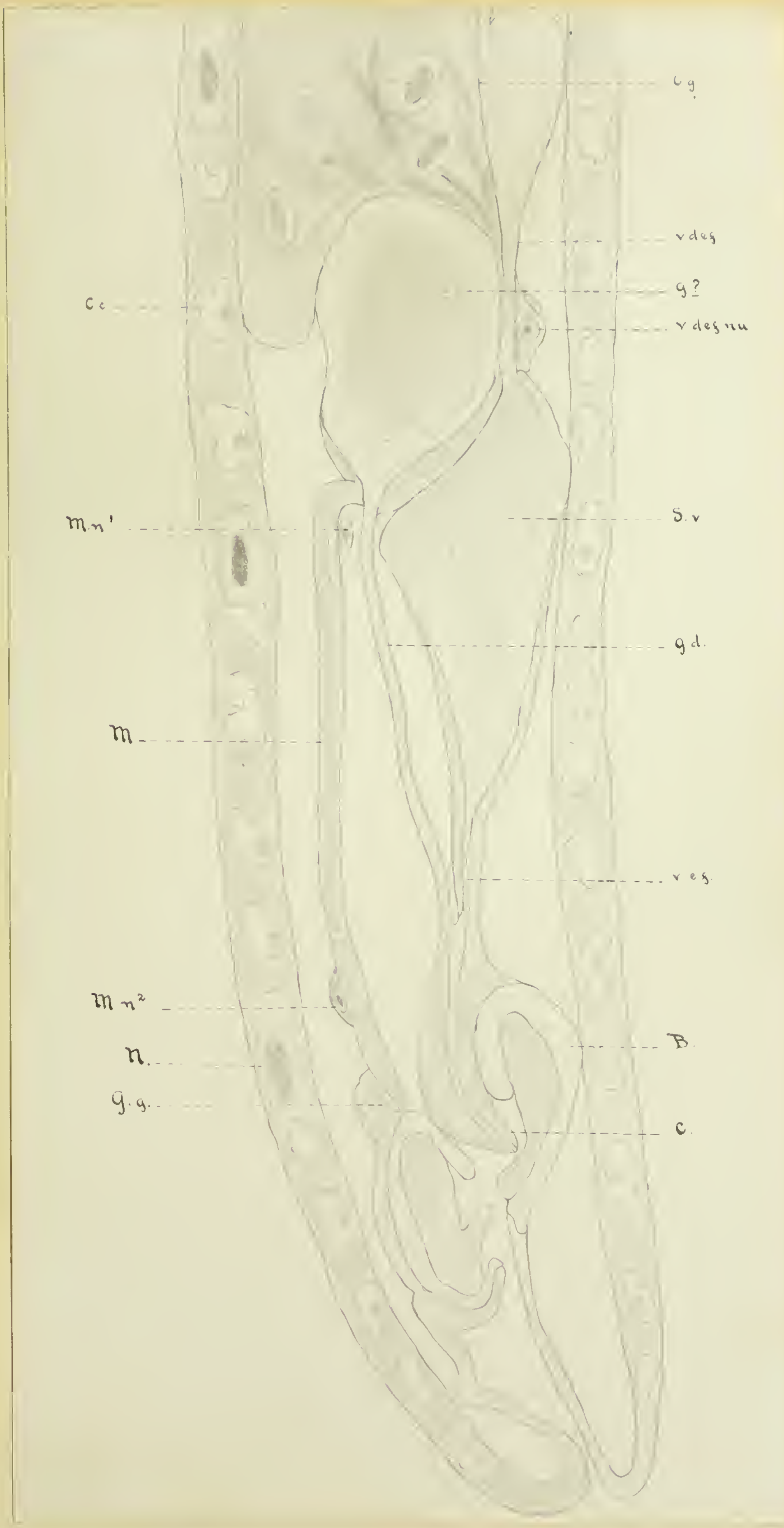
N. Subcuticular nuclei.

S.v. S eminal vesicle.

v.def. vas deferens.

v.def. n. Nuclei along side of vas deferens.

v.ef. vas eferens.



c g.
v deg
C c
g ?
v deg nu
M.n'
S. v
g d.
M
v eg.
M n^2
N.
B.
G. g.
C.

Figure 3.

Reconstruction of posterior end of female, showing genital tract. Enlarged 333 diameters.

A.&C.-G. Nuclei whose location is explained in text.

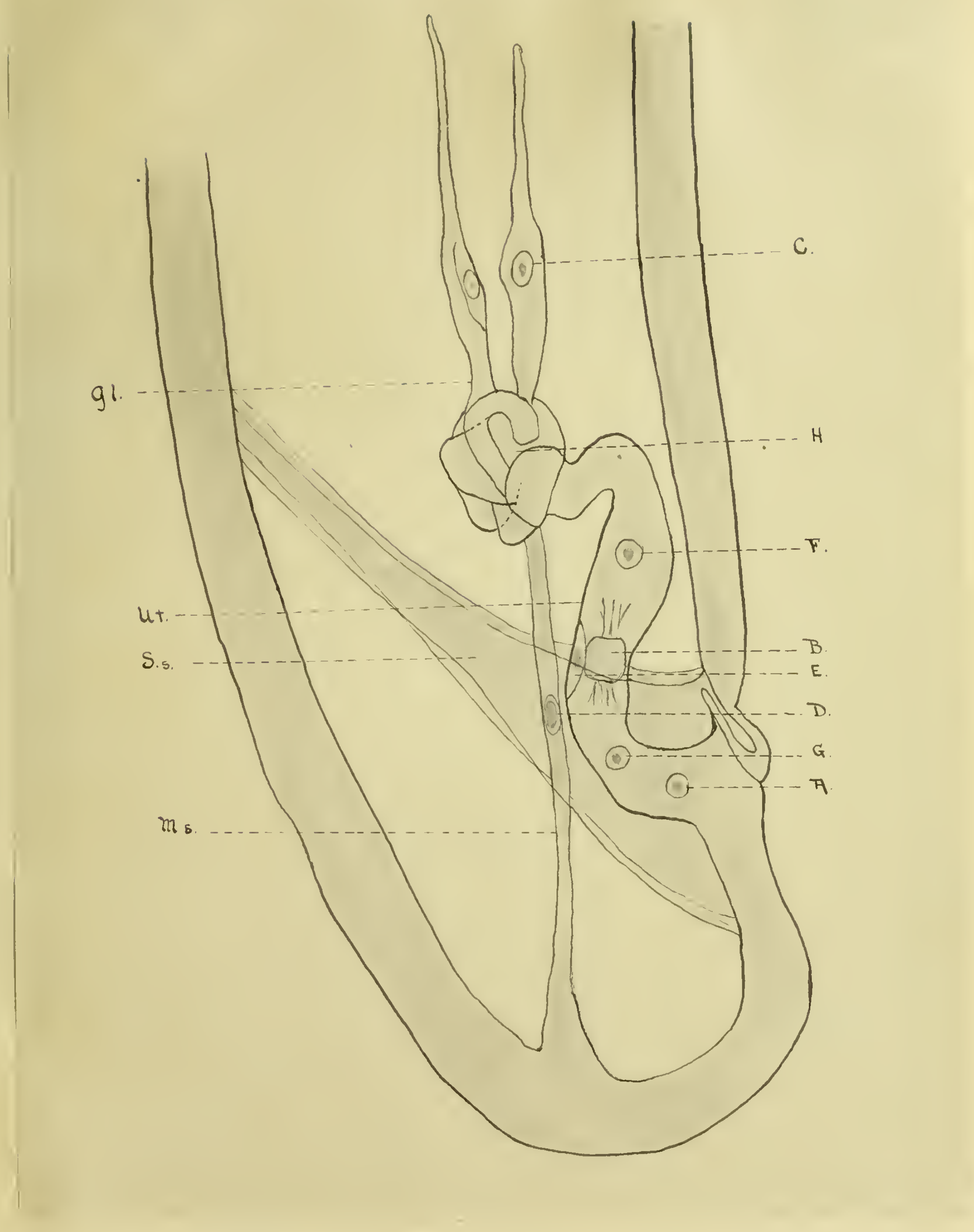
B. Sphincter.

Gl. Bell or Glocke.

H. Oviduct.

S.s. Supporting tissue sheath.

Ut. Uterus.



C.
gl.
H
F.
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Ms.